Sequencing Cas9 *in vitro* assay:

Random variant plasmid library:

= PvuI cleavage site

= Cas9 cleavage site

*PvuI linearizes the library plasmids
Plasmid size: 4.1 kb

Non-amplification based Cas9 *in vitro* assay:

unc-22A C11G

pb_FU-63

unc-22A WT

pb_FU-1515

*PvuI makes two cuts on each of the plas-mids

unc-22 C11G band sizes: 2377, 1045
WT band sizes: 2261, 1045

Incubate gRNA & Cas9:

5'
20 nt
linker loop
3'
crRNA-tracrRNA chimera

unc-22A C11G

unc-22A WT

PCR amplify and run library samples on Miseq:

Run samples on agarose gel :

Library + PvuI: 4.1 kb
Library + PvuI + Cas9: 2 x ~2kb
pb_FU-63 + PvuI: 2337 bps
pb_FU-1515 +PvuI: 2261 bps
pb_FU-63+PvuI+Cas9: 1982 bps
pb_FU-1515+PvuI+Cas9: 1933 bps
pb_FU-63+PvuI & -1515: 1045 bps
pb_FU-63 & -1515+PvuI: ~400 bps